Prevalence of clinical and subclinical mastitis and quality of milk on smallholder dairy farms in Tanzania

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ABSTRACT

A cross sectional study was conducted during October and November 2006 on 69 smallholder dairy farms with lactating cows in Mvomero and Njombe districts Tanzania, to determine the prevalence of mastitis and to assess the milk quality on the study farms. Clinical mastitis was investigated using clinical changes of udder and milk at animal level. Cow-side California Mastitis Test (CMT) and microbiological cultures were used to assess subclinical mastitis at quarter level. Milk quality was determined on bulk milk samples at herd level using alcohol and acidity tests, butter fat content, total solids, ash content as well as Delvotest® for antimicrobial residues. Overall prevalence of clinical mastitis at herd level in both districts was 21.7 % (n = 69). Based on CMT, prevalence of subclinical mastitis at animal level was 51.6 % (n = 91). Prevalence of bacterial isolates at animal level was 35.2 % (n=91) while for fungal it was 16.7 % (n=90). Based on CMT results, prevalence of subclinical mastitis at quarter level was 30 % (n = 353), while for bacteria and fungi it was 16 % and 6 % respectively. Contamination of milk with antimicrobial residues was 4.5 % (n =67). The milk quality parameters for most of the milk samples were within acceptable levels. Findings in this study have demonstrated high prevalence of subclinical mastitis that may contribute to low productivity of dairy cattle in both districts. About 20 % of CMT subclinical cases had no involvement of microbial pathogens that suggested the need for minimal interventions with antimicrobial agents. These findings call for use of udder disinfectants and improved milking hygiene as intervention strategies to control mastitis on the smallholder dairy farms in Tanzania.

Keywords: antimicrobial residues, bacteria, CMT, fungi, intramammary infection, smallholder dairy farms, udder health.

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INTRODUCTION

Smallholder dairy farming has a significant role in the alleviation of poverty and reduction of malnutrition in Tanzania. Dairy animals provide regular household income, employment, and nutritious food¹. However, the dairy farming suffers from constraints that limit realisation of this potential. For instance, farmers in Tanzania continue to experience suboptimal performance of their animals due to factors such as diseases, poor management practices and suboptimal feeding regimens^{6,14}.

forms is the main disease that affects milk production 12,16,17. Apart from lowered productivity, mastitis also reduces milk quality as a result of changes in milk composition and also by contamination of milk by drugs used for treatment of the disease¹⁸. The quality of milk may also be impaired by a number of other factors such as adulteration, spoilage from poor storage and contamination during and after milking⁵. Addition of water by dishonest middlemen and animal attendants is also among the most common forms of adulteration. Furthermore, being high in fat content, milk absorbs environmental odours easily. Constraints in milk marketing also limit optimal productivity on the smallholder dairy farms. Lack of processing of milk into better paid products further minimises the accrued income from milk production.

In view of the high prevalence of factors that contribute to poor milk quality, milk

Mastitis in both clinical and subclinical

testing and quality controls are essential components of milk production. Thus, in cases where mastitis is highly prevalent and milk on the market is of reduced quality, farmers fail to realise the expected benefit from dairy production. In order for any processor to make good dairy products, raw milk of good quality is crucial. However, a milk processor or a handler will only be assured of the quality of raw milk if certain basic quality tests are carried out at various stages from production to consumers. The aim of the study was to firstly aquire information on the magnitude of clinical mastitis and establish risk factors for the disease. Secondly, the prevalence of subclinical mastitis was assessed by CMT and microbiological milk cultures. Thirdly, milk quality was evaluated based on physico-chemical parame-

MATERIALS AND METHODS

This study was conducted during October and November 2006. A total of 69 smallholder dairy farms with lactating cows in 9 villages participated in the study, of which 28 (40.6 %) and 41 (59.4 %) were from Mvomero and Njombe districts, respectively. Because of the small number of lactating cows on participating farms (i.e. 1.4 ± 0.6 , mean \pm SD), all lactating cows on study farms were included in the study. Thus in total 35 and 56 cows were examined in Mvomero and Njombe districts, respectively. During farm visits, a structured questionnaire was used to collect information at herd and animal levels regarding milk yield, milking practices, occurrence of clinical mastitis, use of post-milking disinfectants and application of dry cow therapy in controlling mastitis. Animal-level information included age (in years), parity, number of months post-calving, milk production records (peak yield and the amount produced on the day of the farm visit), and health status of the animal on the day of the farm visit. Observational assessment was also made on the hygiene of animal stables. In total, 91 lactating cows from 69 smallholder farms were scored for body condition (BCS) as previously described¹⁹ and clinically examined for presence of

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clinical mastitis as evidenced by changes in the udder and milk¹⁸. Presence of subclinical mastitis in each quarter was checked using cow-side CMT as previously described16. Milk was collected aseptically into sterile receptacles from each teat for microbiological examination and samples were stored in cool boxes at 3–5°C for 12 hours during shipment to the laboratory at Sokoine University of Agriculture (SUA), Microbiology laboratory in the Faculty of Veterinary Medicine, where they were cultured within 1 hour of arrival for isolation of aerobic bacteria and fungi using standard procedures². In addition, pooled milk samples from all study farms in Njombe district were collected at each farm after milking was complete and tested for the presence of antimicrobial residues using Delvotest®9 as well as for physico-chemical characteristics using the alcohol test, lactometer, acidity, total solids, butter fat and ash⁷. For the purpose of this test about 30 m ℓ of milk samples were collected from the common container with pooled milk from all milked animals. During sample collection alcohol, lactometer and acidity tests were performed at the farm. After these tests, samples were stored in a cool box at 3-5°C and shipped to SUA for analysis of total solids, butter fat and ash.

Data analysis

Data were entered in Epi Info databases⁴ and the association between mastitis and risk factors was determined by logistic regression analysis using Epi Info³. This involved univariate and multivariate analyses whereby the associations between the outcome variable (subclinical mastitis defined by the CMT and microbiological culture) and explanatory variables (potential risk factors at herd and animal levels) were assessed. Explanatory variables with P < 0.25 were tested in the multivariate analysis. Fitting of multivariate models was carried out using a combination of forward inclusion and backward elimination processes until a stable final model for each outcome variable was obtained. For other variables related to milk quality, descriptive statistics for different variables were computed. Comparison of proportions in different groups was done using the chi-square test whereas the analysis of variance (ANOVA) was adopted to compare difference in means of continuous variables on data that were normally distributed11. For data that were not normally distributed, modes and range are reported.

Definition of outcome variables

Subclinical mastitis defined by CMT
The CMT results were judged on

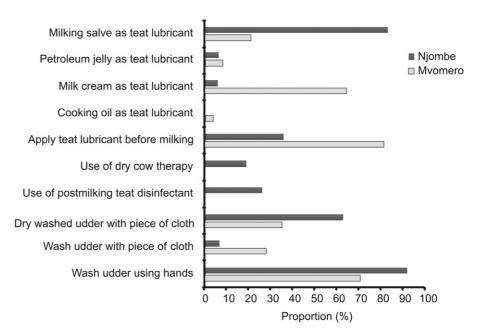


Fig. 1: Proportion (%) of different pre- and post-milking practices on smallholder dairy farms in the Njombe (n = 41) and Mvomero districts (n = 28).

5-point scale (negative, trace, 1+, 2+ or 3+) and a quarter was defined as CMT-positive if it had a score of $\geq 2+$. A cow was defined as CMT-positive when it had at least 1 quarter with a CMT-positive score of $\geq 2+$.

Subclinical mastitis defined by microbiological cultures

A quarter was defined as positive when a pathogen (bacterium or fungus) was isolated during microbiological culture. A cow was considered positive when at least 1 quarter milk sample had a pathogen isolated.

Mastitis defined by clinical signs

A cow was considered to have clinical mastitis if she showed changes in milk including presence of pus, clots, flakes or blood and/or changes of the udder including swollen or painful quarter that were evident on the day of the farm visit. For the reported cases of clinical mastitis, cows were considered to have had clinical mastitis in the past if the farmer reported having observed similar changes in milk and/or udder during the year prior to the present study.

RESULTS

Dairy farms

Different practices were applied by farmers before, during and after milking in the 2 districts (Fig. 1). All farmers in both districts washed their hands and the whole udder with cold water before milking. Washing the udder was done using either bare hands or a piece of cloth. The proportion of farmers that washed the udder with bare hands was significantly

higher (P < 0.05) in Njombe (92.5 %) than in Mvomero (71.4 %). After washing, drying of udder was done using bare hands or a piece of cloth. The proportion of farmers that dried the washed udder with a piece of cloth was significantly higher (P < 0.05) in Njombe (63.4 %) than in Mvomero (35.7 %). Use of post-milking teat disinfectants and dry cow therapy was practised by smallholder dairy farmers in Njombe only at 26.8 % and 19.5 %, respectively. Different types of teat lubricants were applied by farmers during milking and included milking salve, milk cream and cooking oil. The proportion of farmers that applied teat lubricant during milking was significantly higher (P < 0.05) in Mvomero (82.1 %) than in Njombe (36.6 %). Whereas milk cream was the main type of lubricant used by farmers in Mvomero, in Njombe milking salve was predominant. Overall, more than 50 % of the milking process was carried out by women. The proportion of women who were involved in milking the cows was significantly higher (P < 0.05) in Njombe (61.0 %) than in Myomero (35.7 %) dis-

The overall herd size (mode) of small-holder dairy farms in Mvomero and Njombe districts was 2 (ranging from 1 to 7) dairy cattle including followers (calves and weaners) per household. The amount of milk produced on the study small-holder dairy farms was 7.7 ± 3.1 litres/cow/day (mean \pm SD). There was no significant difference in the average milk production per cow per day in Mvomero (7.9 ± 2.9) and Njombe (7.5 ± 3.3) districts. The average body condition score for lactating cows was 1.7 ± 0.5 with cows in Mvomero district having higher condi-

Table 1: Distribution of mastitis pathogens by California mastitis test (CMT) status.

Bacterial isolates	CMT status				% Samples	% Isolates	
	_	+	2+	3+	Total	(n = 58)	$(n = 54)^*$
Contamination	3	0	0	1	4	6.9	_
Bacillus spp.	0	1	0	0	1	1.7	1.9
Escherichia coli	1	0	0	0	1	1.7	1.9
Staphylococcus aureus	5	3	2	1	11	19	20.3
Staphylococcus sp.	3	9	15	13	40	69	74
Streptococcus agalactiae	1	0	0	0	1	1.7	1.9
Total number of isolates	13	13	17	15	58	100	100

^{*}Excludes 4 samples that were contaminated.

tion scores (1.9 \pm 0.5) than those in Njombe district (1.5 \pm 0.5), a difference that was significant (P < 0.05). Of the 69 households with lactating cows, 8 (11.6 %) had cows dried off during the field visit. In addition, 33 (47.8 %) households out of 69 fed their calves by bucket while residual calf suckling was practiced by 36 (52.2 %). Overall, there was no surveillance programme for mastitis at farm level in either district.

Herd-level prevalence of clinical mastitis

Reported overall prevalence of clinical mastitis at herd level in the study villages was 21.7 % (n=69 households) and all villages except 1 had at least 1 farm with case(s) of clinical mastitis within the period of 1 year before this study. Overall, 7 inactive (blind) quarters were observed only in a few animals, which included 1 fore left quarter and 2 hind left quarters. Among the right quarters 2 front and 2 hind teats were also inactive.

Cow- and quarter-level prevalence of subclinical mastitis

Overall, out of the 91 CMT-tested cows on 69 smallholder dairy farms, 51.6 % tested positive for subclinical mastitis and the proportion of subclinically infected cows per village ranged from 33.3 % to 100 %.

Overall prevalence of subclinical mastitis based on bacterial isolation at animal level was 35.2 % and bacteria that were isolated included coagulase-negative staphylococci (74 %), Staphylococcus aureus (20.4 %), Streptococcus agalactiae (1.9 %), Bacillus spp. (1.9 %) and E. coli (1.9 %). Based on fungal isolation, prevalence of subclinical mastitis was 16.7 % and the isolates included yeast (95 %) and cryptococci (5 %) (Table 1).

Risk factors for subclinical mastitis defined by CMT and microbiological culture-positive cows

The distribution of quarters that were positive on CMT and microbiological culture is shown in Fig. 2 and the results from the univariate analysis for subclinical

mastitis defined by CMT results are summarised in Table 2. There was no significant association between CMT-positive quarters and peak cow's milk yield, body condition score, months post partum, district (i.e. Mvomero vs Njombe), use of cloths to wash teats before milking and number of cows (lactating and dried off). The final model for the CMT-positive quarters indicated that a CMT-positive reaction was positively related to isolation of bacterial pathogens (OR = 8.35) and negatively related to total milk produced on each farm ($\beta = 0.098$). However, the number of CMT positively infected quarters was significantly higher (P < 0.05) than quarters infected with microbial pathogens alone or microbial pathogens in combination with CMT results (Figs 2, 3). Results of univariate analysis of associations between risk factors and bacterial culture-positive cows are shown in Table 3. Factors that did not demonstrate significant association with bacterial culture results included parity, age category,

peak milk yield of cows, history of clinical mastitis, body condition score, district (Mvomero vs Njombe), use of cloths to wash and dry teats, number of dried off cows on farm, herd size, use of teat lubricants, total milk yield per farm per day and the principal milker on the study farm. The final model for the positive bacterial culture included CMT-positive cows (OR = 8.87), fungal culture-positive results (OR = 4.25) and the number of lactating cows present on a farm on the day of visit (β = 0.907).

Milk quality based on physicochemical and antimicrobial residue analysis

Crosses of Ayrshire, Friesian and Boran were the only breeds of cattle kept by farmers in both districts. In Njombe district where milk quality was assessed, the breed distribution was 38 %, 48.8 % and 12.2 % for Ayrshire, Friesian and Boran breeds respectively. The overall results for physico-chemical analysis of bulk milk

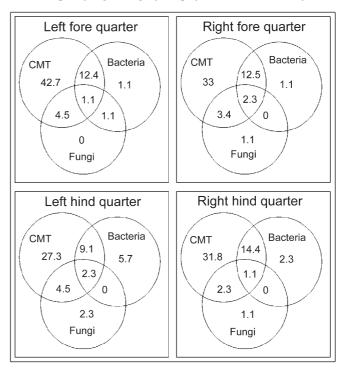


Fig. 2: Distribution (%) of quarters that tested positive for CMT and microbial culture in Njombe and Mvomero districts (n = 357).

Table 2: Association of California mastitis test (CMT) and explanatory variables (P < 0.25) in Mvomero and Njombe districts.

Variable	OR (95 % CI)	B (SE)	Z-statistic	P-value			
Univariate analysis							
Current milk yield at cow level	0.91 (0.79-1.06)	-0.093 (0.076)	-1.230	0.2189			
Bacterial culture-positive at quarter level	6.176 (1.69-22.62)	1.821 (0.662)	2.749	0.006			
Fungal culture-positive at quarter level	3.06 (0.64-14.64)	1.118 (0.799)	1.399	0.1616			
Cow had clinical mastitis in the past	3.06 (0.64-14.64)	1.118 (0.799)	1.399	0.1616			
Use cloth to dry teats before milking at herd level	0.55 (0.21-1.40)	-0.605 (0.481)	-1.258	0.2084			
Herd size	0.79 (0.61-1.04)	-0.230 (0.137)	-1.686	0.0918			
Use teat lubricant before milking	0.47 (0.19-1.20)	-0.749 (0.475)	-1.578	0.1146			
Total milk produced on the farm	0.94 (0.87-1.00)	-0.067 (0.036)	-1.854	0.0637			
Women or attendant as principal milker	0.35 (0.07–1.83)	-1.042 (0.841)	-1.239	0.2155			
Final model for multivariate analysis of CMT-positive animals							
Bacterial culture-positive at quarter level	8.35 (2.06-33.81)	2.123 (0.714)	2.973	0.0030			
Total milk produced on the farm	0.91 (0.835–0.986)	-0.098 (0.043)	-2.298	0.0215			

Table 3: Association of bacterial culture-positive results and explanatory variables (P < 0.25) in Mvomero and Njombe districts.

Variable	OR (95 % CI)	B (SE)	Z-statistic	<i>P</i> -value
Univariate analysis				_
Current milk yield at animal level	0.91 (0.78-1.05)	-0.099 (0.074)	-1.348	0.1777
CMT-positive cow	6.18 (1.69–22.62)	1.821 (0.662)	2.749	0.0060
Fungal culture-positive at animal level	4.82 (1.48-15.73)	1.572 (0.604)	2.605	0.0092
Months post-calving	1.13 (1.02–1.25)	0.124 (0.052)	2.390	0.0169
Number of lactating cows on the farm	1.83 (1.05–3.19)	0.602 (0.285)	2.116	0.0344
Final model for bacterial culture-positive				
CMT-positive cow	8.87 (1.99-39.48)	2.182 (0.762)	2.864	0.0042
Fungal culture-positive at animal level	4.25 (1.17–15.39)	1.446 (0.657)	2.202	0.0276
Number of lactating cows on the farm	2.48 (1.24–4.96)	0.907 (0.354)	2.563	0.0104

samples are shown in Fig. 3 and Table 4. Overall, 27.5 % (range 10-50 %) of milk samples tested were positive in the alcohol test. Although all study villages in Njombe district had positive samples in the alcohol test, the highest proportion was in Kichiwa village (Fig. 4). Acidity was significantly higher (P < 0.01) in milk samples that were collected from Kichiwa village. Milk density based on lactometer reading, butter fat content, total solids and ash contents were within the normal range (Table 4). Since butter fat content can vary between breeds of dairy cattle, further analysis of data with breed factor considerations was made. The butter fat content (%) in Avrshire, Friesian and Boran breeds was 3.01 ± 0.03 , 3.12 ± 0.005 and 2.42 ± 0.02 respectively. However, the difference between breeds was not significant (P < 0.05). As demonstrated by the Delvotest®SP, contamination of milk

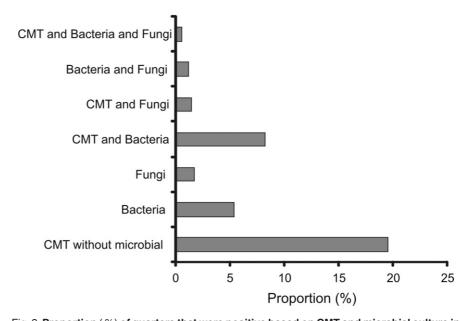


Fig. 3: Proportion (%) of quarters that were positive based on CMT and microbial culture in Njombe and Mvomero districts (n=375).

Table 4: Milk quality parameters for milk samples from Njombe district.

Village	Farms n	Acidity (%) mean ± SD (range)	Butter fat (%) mean ± SD (range)	Lactometer mean ± SD (range)	Total solids (%) mean ± SD (range)	Ash (%) mean ± SD (range)
Overall	40	0.21 ± 0.07	2.99 ± 0.86	26.6 ± 2	11.3 ±1.7	0.65 ± 0.09
		(0.15-0.44)	(1–5)	(20.8-30.6)	(8.6-19.8)	(0.47-0.84)
Normal values*		0.15-0.17	≥ 3.3	26–32	12.5–13	0.7–0.8

^{*}Source: ILCA, 1988

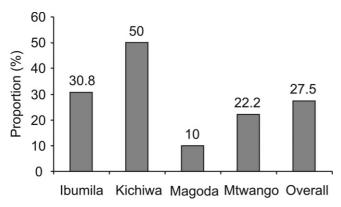


Fig. 4: Proportion of milk samples that tested positive in the alcohol test in study villages in Njombe district (n = 41).

with antimicrobial residues was found in 4.5% of samples.

DISCUSSION

The standard of milking hygiene was poor on the majority of the study farms in both districts (Fig. 1). Mastitis preventive measures, such as the use of udder disinfectants and dry cow therapy, were only applied by a few farmers in Njombe. The peak and level of milk production were generally low for the dairy animals in both districts. Overall, comparable herd and animal-level results were observed. Thus, data are reported and discussed without stratification by village and district, assuming similar causes of mastitis and low milk yield in smallholder dairy cows in both districts.

Prevalence of clinical mastitis as reported by farmers and demonstrated in this study was low, a finding that was similar to other studies 10,13,16. Most smallholder dairy farmers in Tanzania are aware of clinical mastitis, but are unaware of and have no knowledge about the subclinical form of the disease 15,16. Clinical cases of mastitis are easily diagnosed by farmers and reported to the veterinary practitioners for treatment with intramammary infusions with potential to kill microbial pathogens. Thus, a number of the mastitis-causing pathogens isolated in this study was most likely due to subclinical mastitis or intramammary infection (IMI), which is defined as an infection with no visible changes in milk or the mammary gland, but with decreased milk production and the presence of bacteria in the secretion where inflammatory changes in the milk can be detected by special tests, such as somatic cell count. Furthermore, a considerable number of pathogens isolated in this investigation could as well be due to colonisation of the teat canal and cistern, with an absence of, or only limited, inflammatory response.

High prevalence of subclinical mastitis on smallholder dairy farms was observed in this study, a finding that was comparable to what was previously reported^{12,16}. Predisposing factors, including improper milking hygiene, lack or improper use of post-milking disinfectants, and application of dry cow therapy by few farmers as observed in this study (Fig. 1), were the likely attributable risk factors to the observed high prevalence of subclinical mastitis.

The bacterial pathogens isolated in the current study were dominated by coagulase-negative staphylococci (Table 1). The preponderance of Staphylococcus species in the study animals has also been observed in other studies in Tanzania 15,16. The dominance of this group of pathogens is possibly as a result of poor milking hygiene as was evidenced in the current study (Fig. 1). According to Blowey and Edmonson¹, coagulasenegative staphylococci commonly colonise the teat end and teat canal only and are difficult to associate with clinical mastitis. Under some circumstances, however, they may lead to raised somatic cell counts and subclinical mastitis. Since they are a contagious and common coloniser of the teat end and teat canal, the use of dry cow therapy and post-milking teat disinfectants are of great value in controlling the disease¹. These control measures, however, were not used by most of farmers that participated in the

In the present study, a subclinical mastitis case was defined as an animal with at least 1 of the teats with a CMT score of $\geq 2+$ and/or microbiological culture-positive results. Most of the animals diagnosed with subclinical mastitis were positive on CMT only and few on CMT and bacterial culture, CMT and fungal culture or CMT and bacterial and fungal culture together (Fig. 2). Overall, about 20 % of the quarters were positive on CMT and very few on microbiological cultures. Animals whose milk samples were only microbial culture-positive and CMT-negative were initially regarded as having IMI rather than mastitis. However, the decision to in-

clude bacterial culture results as an outcome variable was based on a strong association between CMT and isolation of mastitis pathogens in this study as well as results of other studies in Tanzania8. Since, microbial colonisation of the teat canal or cistern can occur without involvement of the udder parenchyma, it is obvious that microbial culture alone cannot be used to confirm cases of subclinical mastitis. These findings demonstrate that CMT and/or microbial culture tests when considered separately are inferior to when both tests are interpreted together in confirming cases of mastitis even at the initial and recovery stages of the disease. According to Blowey and Edmondson¹, storage of milk samples at 4°C for 72 hours before culture has no significant effect on the isolation rate of the microbial pathogens. Since during this study collected samples were stored at 3-5°C and cultured within 12-13 hours, it is unlikely that this contributed to less sensitivity of culture than CMT mastitis detection methods.

Findings in this study have demonstrated a high prevalence of fungal infections at herd and quarter levels (Fig. 3). Although fungal infection can be associated with prolonged use of antibiotics, this was not the case in the study areas since the Delvotest® indicated very low levels of antimicrobial residues in milk samples. In addition, farm records did not show high usage of antimicrobial agents for the past year. Thus, the high level of fungal infection observed could be of environmental origin, particularly due to poor water quality and poor hygiene of stables¹. Thus, in order to reduce environmental mastitis, pre-milking disinfectants could be useful¹.

Presence of antimicrobial residues in milk samples was low, suggesting that antimicrobial residues may not be a problem in the smallholder dairy farming sector, a finding which was similar to what was previously reported9. Based on the results from alcohol and acidity tests, milk in only 1 village in Njombe district (Kichiwa village) was of poor quality. Since farmers in this village have no milk cooling and storage facilities (refrigerators and deep freezers) and the fact that when the study was conducted farmers were not selling milk to the processing plant, mixing of evening and morning milk was a common practice. This might have been the cause for the high acidity of milk observed as well as the high proportion of milk samples that were positive in the alcohol test. Thus, training of farmers on factors leading to poor quality of milk and provision of reliable cooling facilities would be relevant.

In conclusion, this study has demonstrated a high prevalence of subclinical mastitis that could be among major constraints that limit optimum productivity of smallholder dairy cattle in Tanzania. Since this form of mastitis is an unfamiliar problem to farmers, and because of its insidious nature, it may be responsible for considerable losses. Thus, if milk production in the study area is to be improved, creation of awareness and training of farmers on the control of mastitis is important. Since the highest proportion of subclinical mastitis cases were positive by CMT without evidence of microbial infections, the intervention strategy should focus on improved management, frequent monitoring of subclinical mastitis in milking cows with CMT and use of teat disinfectants; with minimal or without treatment with antimicrobial agents. This approach would ensure minimal exposure of milk consumers to antimicrobials and the associated residues. In addition, this will minimise misuse of antimicrobial agents and the associated resistance which is now an emerging problem. Overall, the quality of most of the milk was of an acceptable standard and contamination of milk with antimicrobial residues was low.

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